Differential effects of an obesogenic post-weaning diet on male and female behaviour in mice

Emily Mort[1], Surina Fordington[1], Sophie Heritage[1], Abigail Fowden[1], Susan Jones[1], and Emily Camm[1]

[1]University of Cambridge

February 17, 2023

Abstract

Obesity is rising globally and is associated with neurodevelopmental and psychiatric disorders among children, adolescents, and young adults. Whether obesity is the cause or the consequence of these disorders remains unclear. To examine the behavioural effects of obesity systematically, locomotion, anxiety, and social behaviour were assessed in male and female C57Bl/6J mice using the open field (OF), elevated plus maze (EPM) and social preference (SP) task. First, the effects of age, sex and prior exposure to the tasks were examined in control mice, before investigating post-weaning consumption of a high fat, high sugar (HFHS) diet commonly consumed in human populations with high rates of obesity. In the OF and EPM, locomotor activity and anxiety-related behaviours were reduced by age in both sexes, but with different sex-specific profiles. Prior exposure to the tasks reduced locomotion in the OF in a sex-specific manner but had little effect on behaviour in the EPM in either sex. The HFHS diet reduced food and calorie intake and increased body mass and fat deposition in both sexes. In the OF, both male and female HFHS mice showed reduced locomotion, whereas, in the EPM, only HFHS female mice displayed reduced anxiety-related behaviours. Both male and female HFHS mice had a significantly higher SP index than controls. Collectively, the findings demonstrate that the behavioural effects of age, prior exposure and of diet-induced obesity all depend on the sex of the mouse. This emphasises the importance of including both sexes when assessing behavioural phenotypes arising from dietary manipulations.

Hosted file

`Mort et al Figures 16eb2023.pptx` available at https://authorea.com/users/587098/articles/624927-differential-effects-of-an-obesogenic-post-weaning-diet-on-male-and-female-behaviour-in-mice

Differential effects of an obesogenic post-weaning diet on male and female behaviour in mice

[1]Emily J. Mort, [1]Surina Fordington, [1]Sophie Heritage, [1]Abigail L. Fowden, [1]Susan Jones*, [1,2]Emily J. Camm*

1. Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3EG, United Kingdom

2. The Ritchie Centre, Hudson Institute of Medical Research, 27-31 Wright Street, Clayton, VIC 3168, Australia

*Joint corresponding and senior authors

sj251@cam.ac.uk

emily.camm@hudson.org.au

Number of pages: 23

Number of Figures: 9

Key words

Sex differences; open field; elevated plus maze; social preference; high fat/ high sugar.

Funding

The work was funded by an MRC STP studentship to Emily J. Mort (Grant number; MR/N013433/1).

Contributor statement

EJC and ALF designed the study; EJC and EJM carried out the experiments; EJM, SF and SH evaluated the behavioural tasks; EJM, ALF, EJC and SJ analysed the data, EJM and SJ created the figures; EJC and SJ wrote the text, all authors reviewed and revised the paper.

Abstract

Obesity is rising globally and is associated with neurodevelopmental and psychiatric disorders among children, adolescents, and young adults. Whether obesity is the cause or the consequence of these disorders remains unclear. To examine the behavioural effects of obesity systematically, locomotion, anxiety, and social behaviour were assessed in male and female C57Bl/6J mice using the open field (OF), elevated plus maze (EPM) and social preference (SP) task. First, the effects of age, sex and prior exposure to the tasks were examined in control mice, before investigating post-weaning consumption of a high fat, high sugar (HFHS) diet commonly consumed in human populations with high rates of obesity. In the OF and EPM, locomotor activity and anxiety-related behaviours were reduced by age in both sexes, but with different sex-specific profiles. Prior exposure to the tasks reduced locomotion in the OF in a sex-specific manner but had little effect on behaviour in the EPM in either sex. The HFHS diet reduced food and calorie intake and increased body mass and fat deposition in both sexes. In the OF, both male and female HFHS mice showed reduced locomotion, whereas, in the EPM, only HFHS female mice displayed reduced anxiety-related behaviours. Both male and female HFHS mice had a significantly higher SP index than controls. Collectively, the findings demonstrate that the behavioural effects of age, prior exposure and of diet-induced obesity all depend on the sex of the mouse. This emphasises the importance of including both sexes when assessing behavioural phenotypes arising from dietary manipulations.

Introduction

The incidence of obesity defined by the World Health Organisation as a body mass index (BMI) of greater than 30kg (weight)/m^2 (height) is increasing rapidly worldwide in both developed and developing countries, largely as a result of dietary changes (Organisation., 2021). In the UK, the incidence of obesity has tripled in the last 50 years with about two-thirds of the adult population now classified as overweight or obese, although the exact figures vary with age and ethnicity (Digital, 2020). Obesity is also increasing in children with approximately 20% of 10-11 year olds classed as obese in 2018 (Digital, 2020). Obesity and overweight are known to be major risk factors for adult cardiovascular and metabolic diseases (Powell-Wiley et al., 2021). They also have a negative impact on behaviour and mental health from childhood onwards with associations between a high BMI and a wide range of behavioural alterations in human epidemiological studies including depression, impaired memory and poorer cognitive performance (Edlow, 2017). In particular, obesity is associated with increased anxiety (Fulton et al., 2022) and risk-taking behaviours in both adolescent and adult human populations (Ratcliff et al., 2011). The specific alterations in behaviour have also been shown to be linked to age and sex in certain instances. However, whether obesity is the direct cause or the consequence of the behavioural changes remains unclear as the prevalence of anxiety disorders increases with obesity while anxiety and risk-taking behaviours predispose individuals to weight gain.

The bidirectional nature of this relationship can be studied more systematically in experimental animals like rodents, as dietary intake and adiposity can be measured precisely in these species. In rodents, obesogenic diets have been shown to both increase and decrease anxiety- and risk-taking behaviours, which may reflect differences in the age of the mice and/or in the dietary regime between the studies (Clark et al., 2022a, Yoshizaki et al., 2020, Haleem and Mahmood, 2021, Lopez-Taboada et al., 2020, Clark et al., 2022b). Often, little information is provided on the dietary composition, specific nutrient and calorie consumption, or on the actual degree of adiposity as body weight is frequently used as a proxy measure of obesity in rodents. Many of the studies have used high fat diets rather than diets high in both fat and sugar more commonly eaten by the increasingly overweight and obese human populations. In addition, the majority of behavioural studies of obesogenic diets in rodents have focussed on males. Because of the possibility of greater variability in female data during the oestrus cycle, few studies have investigated the behavioural consequences of obesogenic diets in female mice. Even fewer have studied both males and females simultaneously using the same dietary and experimental regime. These omissions make it difficult to determine if the behavioural changes seen in previous studies of calorie-dense diets in mice are attributable to obesity *per se* or reflect other dietary factors known to alter behaviour such as protein deficiency (Lopez-Taboada et al., 2020, Clark et al., 2022b).

Further, to limit the number of animals used, specific behaviour tests have often been repeated in the same animal. Prior experience to a behavioural task may shift the emotional state of the animal, and lead to

potential masking, or exaggeration, of the true behaviour when re-exposed to the task (Rodgers and Shepherd, 1993, Bertoglio and Carobrez, 2000). Previous studies have shown altered behaviour when the periods between tasks are short (1-3 days), while longer (3–4 weeks) periods before re-testing appear to minimise any alterations (File et al., 1990, File and Zangrossi, 1993, Schneider et al., 2011). Moreover, although many studies have investigated changes in behaviour and cognition with age over narrow windows of time, some have employed large age ranges between experimental groups (e.g. several months, (Lalonde and Strazielle, 2009)) or classified a large age range as one experimental cohort (e.g. 8-12 months, (Shoji et al., 2016)). Levels of anxiety-related and social behaviour have been shown to change between adolescence and adulthood when assessed using common tasks such as the open field (OF), elevated plus maze (EPM), and social preference (SP) tasks (Shoji et al., 2016, Macri et al., 2003, Lalonde and Strazielle, 2009, Nolte et al., 2019). Consequently, the pattern of behavioural change with age requires further investigation even on a standard rodent diet.

The first aim of the current study was to assess behavioural changes associated with aging, sex, and prior experience in mice to provide baseline data before investigating the effects of an obesogenic diet. Gross locomotion and anxiety-like and social behaviours were evaluated in male and female C57Bl/6J mice using the OF, the EPM and SP tasks. The OF and EPM tasks were performed at three key stages of the life course - the boundary between late adolescence and early adulthood (8-9 weeks), early adulthood (11-13 weeks), and middle adulthood (20-22 weeks). The age range within the groups was kept narrow to obtain precise behavioural measures at each life stage. At 20-22 weeks, the mice are still in good health, thus any behavioural changes up to this age should reflect normal ageing and not pathological processes. The effect of prior experience on behavioural outcomes was determined in mice initially tested at 8-9 weeks of age, and then again at 11-13 weeks. The second, and main aim, of the study was to investigate the consequences of consuming a bespoke high fat/high sugar (HFHS) diet on locomotor, anxiety and social behaviours in both male and female mice at 11-13 weeks of age in relation to their calorie and protein intake, somatic growth and fat deposition.

2. Methods

Experimental Design

All animal experimentation was carried out under the UK Home Office Animals (Scientific Procedures) Act 1986, following ethical review by the University of Cambridge. A total of 186 C57Bl/6J mice were used in this study. The initial animals were purchased commercially (Charles River, Margate, UK) but, thereafter, the mice were bred in-house. They were group housed by sex (n=2-5 mice per cage) under a 12:12 h dark/light photocycle with ad *libitum* access to food and water. The majority of the animals studied (n = 136) were fed a standard rodent diet (RM3, Special Dietary Services [SDS], Witham, UK; 11%kcal fat, 62%kcal carbohydrate of which 7%kcal is simple sugar, 27%kcal protein and a water content of 10%). The remainder were fed a customised high fat, high sugar (HFHS) diet from weaning at 21 days of postnatal age to induce increased adiposity in adulthood. The HFHS diet was made by combining high-fat diet pellets (D12451 diet, Research Diets Inc., Denmark) with condensed milk (Carnation, Nestle, Gatwick, UK) and water to form patties that were baked at 55°C for 46–48 h, as described previously (Napso et al., 2022). The final nutritional composition of the HFHS diet was 38%kcal fat, 45%kcal carbohydrate of which 33%kcal was simple sugars, 17%kcal protein, with a water content of 12%. The HFHS and standard diets were replenished every 48h during the period of the study to ensure palatability.

In order to assess the effects of age and sex on behavioural outcomes, groups of mice fed the standard diet were examined at 3 different ages: 8-9 weeks (n=13 females, n=13 males), 11-13 weeks (n= 12 females, n=15 males) and 20-22 weeks (n=5 females, n=18 males). With no previous exposure to any behavioural testing at each age, these mice were classified as 'naïve'. To test the consequences of prior exposure to behavioural testing, the mice studied at 8-9 weeks were tested again with the same behavioural tasks three to four weeks later with the repeat data compared to that of the naïve animals at 11-13 weeks of age. The mice retested at 11-13 weeks were classified as 'exposed'. At the end of the experimental period, the mice used to assess the effects of age, sex and repeated exposure on behavioural outcomes were killed by cervical dislocation.

To determine the impact of an obesogenic diet on mouse behaviour, male and female mice were fed the HFHS diet (n=26 females, n=25 males) for 8-9 weeks before beginning behavioural testing at 11-13 weeks and compared to a control cohort of 11-13 week old mice fed the standard chow (n=36 females, n=31 males). To measure calorie and protein intake, food intake on the two diets was measured weekly from week 9 to week 13 for a subset of the cages and expressed as intake per day per gram of the total weight of mice in each cage. Body weight of the individual mice was also monitored weekly from weaning to the end of the experimental period to measure their weight gain. After finishing behavioural testing at 14 weeks, a subset of the females and males in the two groups were killed by administration of a lethal dose of anaesthetic (20mg/10g, Merial-Euthatal, Coventrus, UK) (Control, n= 20 females; n=15 males; HFHS, n=13 females, n=13 males). These mice

were used to quantify total body fat and lean mass by Dual Energy X-ray Absorptiometry (DEXA) scanning (Lunar PIXImus densitometer; GE Healthcare) and/or to measure the weights of the individual fat deposits and body organs. The remainder of the mice in the dietary study were killed by cervical dislocation.

Behavioural testing

The majority of the mice carried out two different behavioural tests at intervals of 3-4 days. For the naive mice, the two tests were the OF and EPM tasks which were repeated in the same order for the exposed cohort. For the dietary comparison, the behavioural tests were the EPM or one of following combinations of tasks: the OF and EPM or the OF and social preference (SP) task. Female mice were tested in proestrus to account for the effect of oestrus cycle on behaviour (Marcondes et al., 2001, Gangitano et al., 2009). Oestrus cycle stage was determined by visual inspection to avoid invasive vaginal smears, which could have stressed the mice before assessment. A ceiling-mounted webcam was used to video each testing session with the recordings analysed manually blinded to the cohort where possible.

Open Field

The OF test assesses novel environment exploration and general locomotor activity, and provides an initial screen for anxiety-related behaviour in rodents (Prut and Belzung, 2003). The OF arena comprised a $50cm^3$ box with sixteen 5x5cm grids on the bottom, allowing for measurement of movement. The box was separated into two zones: the periphery and the four grids in the centre, to allow assessment of thigmotaxis, the natural tendency of rodents to stay close to walls when traversing an open environment. Mice were placed in the centre of the box and allowed to freely explore the arena for 10 minutes. If a mouse crossed at the point where two lines intersected, such as the corner of one of the grid squares, it was only counted as one line. A mouse was considered to have entered the central zone once all four paws had crossed the boundary; as soon as any paws entered the periphery the mouse was no longer counted as being within the centre. The number of floor grid lines crossed was used as a measure of locomotion. The time spent in the centre and periphery, and the total number of rears were also quantified as a measure of exploration and anxiety (Sturman et al., 2018).

Elevated Plus Maze

The EPM is a widely used behavioural assay for rodents that has been validated to assess anxiety, locomotion, exploration and risk-taking behaviours (Walf and Frye, 2007). It is considered the gold standard method for the evaluation of anxious-type behaviour in rodents. The EPM used in the current study consisted of 65cm long open and closed arms fixed in a plus-formation, with 15cm high walls. The maze was elevated 52cm from the ground. Each mouse was placed in one end of the closed arm facing the wall and allowed to freely explore the maze for 5 minutes, after which it was returned to its home cage. A mouse was considered as having entered an area when all four paws had crossed the boundary and was classified as having left that area as

soon as any paw crossed back over the boundary. The following measures were quantified: open arm time, number of full entries to the open and closed arm, and number of times the mouse reached the end of the open arm (end open arm exploration). The total number of vertical rears was also quantified.

Social Preference

The SP task (Moy et al., 2004) comprises a three-chambered box, with containers placed in the two outer chambers that can house mice. Test mice were habituated to the three-chambered arena for 15 minutes. Empty containers were then added to the arena and the mice were allowed to explore for a further 30 minutes, thereby removing the novelty of the environment so that the focus remained on the contents of the container, rather than the containers themselves driving exploration. The SP test commenced after the habituation period. The position of the containers was balanced between the diet groups. After 5 minutes of habituation for the unfamiliar sex- and strain-matched mouse (intruder), the test mouse was placed into the middle chamber and allowed to freely explore for 10 minutes. Interaction time with the intruder (social stimulus -TS) and non-social stimulus (TNS) was quantified. The social preference index was calculated using the following formula:

$$\text{Social Preference Index} = (TS-TNS)/(TS+TNS)$$

Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 9.4.1 for Windows, GraphPad Software, San Diego, CA, USA). A Shapiro–Wilk normality test was used to examine if data were normally distributed. Mice were deemed to be statistical outliers and excluded if their mean was more than two standard errors from the group mean in three or more measures. Data are presented as mean ± standard error of the mean (SEM). A t-test or Mann–Whitney non-parametric test was used, as appropriate, to compare biometric and behavioural measures between two groups (naïve versus exposed, control versus HFHS). A one-way ANOVA followed by a Dunnett's *post hoc* test or Kruskal-Wallis with Dunn's *post hoc* test was used to compare the effects of age (8-9, 11-13 and 20-22 weeks of age) on behavioural outcome measures. A mixed effects analysis or two-way ANOVA followed by a Sidak's *post hoc* test was used to compare the effects of treatment and age on food intake and body mass. Statistical significance was accepted as $p \leq 0.05$.

Results

Effect of age and sex on locomotor and anxiety-related behaviour

In the OF arena (Figure 1), both age and sex affected locomotion and anxiety-related behaviour over the period from 8 to 22 weeks of age. Specifically, the age of mice at testing had a significant effect on the number of lines crossed in the OF, an index of locomotion (Figure 1A-C). With increasing age, there was a reduction in the number of lines crossed in both male (Figure 1B) and female mice (Figure 1C), although with a different age profile in each sex with males showing a decrease in lines crossed between 11-13 and 20-22 weeks while females showed a decrease in lines crossed from 8-9 weeks to 20-22 weeks (Figure 1B & C). The number of entries into the centre of the OF, the most anxiogenic area of the arena (Figure 1D-E), decreased significantly with age in the males (Figure 2E), but not in the females (Figure 1F). Age had no effect on the time spent in the centre (Figure 1G-I) in either sex (males, p=0.5, Kruskal-Wallis; females, p=0.72, Kruskal-Wallis). Total rearing in the OF (Figure 1J-L) decreased significantly with age in male but not female mice. Overall, the data suggest that age of testing influenced locomotor activity in both sexes but affected anxiety-related behaviour and rearing only in males. These findings demonstrate clear differences in behaviour in the OF between 8-22 weeks of age.

Age and sex were also significant influences on behaviour in the EPM between 8 and 22 weeks of age (Figure 2). With both sexes combined, there was a significant effect of age on the percentage time spent in the open arm of the maze, with significantly less time (percentage of the total testing time) in the open arm at 11-13 weeks than at the other two ages (Figure 2A). This suggests that anxiety in the EPM was maximal at 11-13 weeks. However, the overall decrease in the percentage of time spent in the open arm between 8-9 weeks and 11-13 weeks of age was due primarily to the females (Figure 2C), as there was no change in this parameter in the males between these ages (Figure 2B). In contrast, the percentage of time spent in the open arm was significantly greater at 20-22 weeks of age than at the earlier ages in male mice but was not significantly different at 20-22 weeks relative to the younger ages in the females. By analysing the sexes separately these data, therefore, reveal a sex-specific difference in anxiety-related behaviour within the EPM with age; female mice show increased anxiety by 11-13 weeks of age, while male mice show less anxiety by 20-22 weeks of age.

With increasing age, the number of full entries into either the closed or open arms of the EPM (Figure 2D-I) decreased in both male (Figure 2E, open arm; Figure 2H, closed arm) and female mice (Figure 2F, open arm; Figure 2I, closed arm), suggesting that both sexes became more stationary whether in the centre or in the closed or open arms as they aged. In addition, there was a significant decrease in the number of explorations to the end of the open arm in the female but not the male mice with ageing (Figure 2K, L. Furthermore, total rearing in the EPM was age and sex-related (Figure 2M-O). In the males (Figure 2N), rearing increased between 8-9 and 11-13 weeks of age followed by a significant decrease between the ages of 11-13 and 20-22 weeks.

Conversely, there was no effect of age on rearing in female mice (Figure 2O). Overall, the data suggest that both males and females show less locomotor activity with age, with female mice showing increased anxiety-related behaviour with age whereas males showed less/no change in anxiety-related behaviour and an increase in total rearing at 11-13 weeks of age.

Effect of repeated exposure to a behavioural task on performance

To determine the effect of sex on prior experience of a particular behavioural task, the mice first tested at 8-9 weeks were re-tested with the same task 3-4 weeks later. In the OF task, male mice (11-13 weeks) that had previously been exposed at 8-9 weeks of age showed a significant reduction in locomotor activity (lines crossed) compared with naïve male mice at 11-13 weeks of age (Figure 3A), whilst no significant changes in anxiety-related measures or rearing were observed (Figure 3 C,E,G). Re-tested female mice showed a significant reduction in the time spent in the centre of the OF (Figure 3F) relative to naïve female mice, but no other significant changes in behaviour (Figure 3A, D, H). These data suggest that, broadly, a 3-4 week period between testing can still influence behaviour, and that the effects of prior exposure differ in male and female mice. In the EPM task (Figure 4), 11-13 week old male and female mice with previous exposure to the maze showed no significant changes in their subsequent behaviour compared to naïve counterparts of the same age, with the exception of rearing, which was decreased in number in male mice (Figure 4I).

Food intake, growth rate and fat deposition on a HFHS diet

In order to determine the effect of increased adiposity on locomotion and on anxiety-related and social behaviours of male and female mice, they were fed a HFHS diet from weaning. A mixed effects analysis was performed to compare the effects of age and diet on food, kilocalorie and protein intake between 10-13 weeks. In male mice (Figure 5A), there was a main effect of diet ($p=0.03$) but not of age ($p=0.13$) on food intake with no interaction between age and diet ($F: 3, 32 = 0.15$, $p=0.93$). In female mice (Figure 5B), there was also a main effect of diet ($p<0.0001$) but not age ($p=0.15$), with no interaction between these two factors in influencing food intake ($F: 3, 39 = 0.24$, $p=0.87$). For kilocalorie intake of the male mice (kcal per day per gram of mouse), there was a main effect of diet ($p=0.0007$) but not age ($p=0.21$), with no interaction (Figure 5C; $F: 3, 29 = 0.18$, $p=0.9$). For female mice (Figure 5D), kilocalorie intake per day per gram of mouse varied significantly with diet ($p<0.0001$), with no interaction between age and diet (Figure 5D; $F: 3, 39 = 0.31$, $p=0.8$). There was also a main effect of diet ($p<0.0001$) on protein intake (grams per day per gram of mouse) in male mice, with no significant interaction between age and diet in either sex (Figure 5E, $F: 3, 32 =0.36$, $p=0.78$). In females, there was a main effect of diet ($p<0.0001$) and age ($p=0.049$), but no significant interaction (Figure 5F; $F: 3, 39 = 1.47$, $p=0.23$).

To establish the effect of the HFHS diet on growth rate and fat deposition, body weight, total body fat mass and individual fat deposit masses were measured. A mixed effects analysis was performed to compare the effects of age and diet on body mass. With male body weight (Figure 6A), there were main effects of both age ($p<0.0001$) and diet ($p=0.0006$), with a significant interaction between the two factors ($F (10, 303) = 16.8$, $p<0.0001$). In the females (Figure 6B), there were also main effects of both age ($p<0.0001$) and diet ($p<0.0001$) with a significant interaction between the two in influencing body weight ($F (10, 298) = 40.3$, $p<0.0001$). The HFHS diet led to an increase in body weight in both sexes, which was significant by 11 weeks in the males, and by 9 weeks in the females, relative to their respective controls. At 14 weeks, total body fat mass (grams) measured by DEXA scanning (Figure 6C, D) was significantly greater in both the male (Fig 6E) and female mice (Fig 6G) fed the HFHS diet compared to their control counterparts, at the expense of lean mass (male, Fig 6F; female, Fig 6H). The weights of the perirenal, retroperitoneal and gonadal fat deposits were also significantly increased in both males and females fed the HFHS diet relative to controls. In males fed the HFHS diet (Figure 6N), the percentage contribution of these individual fat deposits to the greater total fat mass increased significantly for each of the three deposits. In contrast, in females fed the HFHS diet, only the retroperitoneal fat accounted for a significantly greater proportion of the increased total fat mass relative to mice fed the control diet (Figure 6P). Fat accumulation, therefore, differed between males and females fed the HFHS diet, with the males gaining proportionally more visceral fat while the females appeared to increase their adiposity more generally.

Effect of HFHS diet on anxiety-related behaviour in male and female mice

Given the observed sex-specific differences in fat distribution and in behaviour with sex, age and prior experience, the effect of the HFHS diet on locomotor, anxiety-related and exploratory behaviour was examined separately in the two sexes aged 11-13 weeks. In the OF test, there was a decrease in locomotion (number of lines crossed) in both sexes fed the HFHS diet compared to their control counterparts (males, Figure 7A; females, Figure 7B). In both males and females, anxiety-related behaviour did not differ significantly with diet either when measured as entries into the centre, (males, Figure 7C; females, Figure 7D) or as duration in the centre (males, Figure 7E; females, Figure 7F). Exploratory rearing in the OF was also unaffected by diet in either male mice (Figure 7G), or female mice (Figure 7H). The data indicate there are no sex-specific differences in the effect of diet on locomotion, anxiety-related behaviour or exploratory behaviour in the OF task.

In the EPM task (Figure 8), there were significant effects of diet primarily in female mice. Relative to control mice, in females fed the HFHS diet, there were significant increases in the percentage time spent in the open arm (Figure 8B), and in the number of full entries into the open arm (Figure 8D), with a concomitant decrease in the number of full entries into the closed arm (Figure 8F). Females fed the HFHS diet also made significantly

more explorations to the end of the open arm (Figure 8H). In male mice fed the HFHS diet, the only significant difference in behaviour observed in the EPM compared to mice fed the control diet was the increased number of explorations to the end of the open arm (Figure 8G). The data indicate marked sex differences in the effect of an obesogenic diet on anxiety-related behaviours in the EPM.

Effect of HFHS diet on social behaviour in male and female mice

There was a significant effect of the HFHS diet on social preference in both male and female mice. Compared with mice fed the control diet, both male and female mice fed the HFHS diet spent an increased percentage of time in the intruder mouse compartment relative to the non-social stimulus compartment (males, Fig 9A; females, Fig 9B). Both male and female mice fed the HFHS diet spent significantly more time interacting with the other mouse compared with the object, measured as the Social Preference Index (males, Fig 9C; females, Fig 9D). These data indicate that diet-induced obesity significantly alters social preference with the HFHS-fed mice spending more time with a novel mouse over an object.

Discussion

The data show that age, sex and re-exposure are all contributing factors to the performance of control-fed wild-type C57BL6/J mice in a range of commonly used rodent behavioural tasks. These factors differentially affected behavioural outcomes during the transition from late adolescence to early adulthood, including locomotor activity and anxiety-related behaviours. Prior experience in the OF arena and EPM resulted in sex-specific effects on locomotion, exploration, and anxiety-related behaviours. Feeding a diet high in fat and sugar reduced food and calorie intake, increased fat deposition and adiposity, and resulted in sex-specific effects on locomotion, anxiety-related and social behaviours. Together, the findings demonstrate that age, sex, and procedural variables, such as repeat testing, are all critical factors that need to be taken into account when designing behavioural studies in mice and in interpreting behavioural phenotypes that result from dietary and other environmental manipulations.

Effect of age and sex on locomotion and anxiety-related behaviour

Although several studies have reported differences in various behaviours between young and aged animals (Shoji et al., 2016, Macri et al., 2003, Lalonde and Strazielle, 2009, Nolte et al., 2019), relatively little is known about the effect of age on behavioural changes at three key stages of the life course- from late adolescence to middle age. Using the OF and EPM behavioural tasks, the current study shows changes in multiple behavioural parameters over this period, including locomotion, exploration, and anxiety-related behaviours. Age significantly reduced locomotion within the OF arena with significantly less movement at 20-22 weeks than earlier in life in both sexes. This result is consistent with previous rodent studies which have reported decreases in locomotion with progressing age through to adulthood (Shoji et al., 2016, Davis et al., 2012). The reduction in locomotive activity in the current study appeared to be progressive with age in the females and to be later in onset in the males. In both sexes, this decline in activity may be explained by an age-related decrease in muscular strength and motor function, or reduced motivation to explore a novel, stressful environment. Mice are not considered aged at 20-22 weeks of age (Hagan, 2017), however, Shoji and colleagues propose that aging from young adulthood to middle age in mice may be associated with decreases in motivation to approach and investigate a novel social environment (Shoji et al., 2016). Further studies are needed to establish whether the reduced locomotion with age is due to a decreased motivation to explore, or to a true reduction in locomotive ability, and the extent to which these factors are sex-specific.

In addition to the locomotive changes, the older male mice showed a decrease in the number of entries into the centre of the OF arena and performed fewer rearings, both of which are indicative of anxiety-related behaviours. In contrast, these age-related changes were not seen in females. In the EPM, older male mice showed an increase in the time spent in the open arms with a significant decrease in closed arm entries, normally suggestive of anti-anxiety behaviour. Whereas female mice showed a decline in both the time spent

in the anxiogenic open arm and the percentage of full entries into the open arm at 11-13 weeks of age, with a reduced number of open arm explorations at 20-22 weeks of age, which could be interpreted as an increase in anxiety-like behaviour. The apparent contradiction in the results on anxiety from the OF and EPM tasks has been reported previously in several inbred and outbred mouse strains (Griebel et al., 2000, Hattori et al., 2012, Miyakawa et al., 2003, Takao et al., 2013). It has been proposed that a higher level of anxiety/high escape response to EPM exposure, could manifest in higher levels of open arm exploration (Hattori et al., 2012, Miyakawa et al., 2003, Takao et al., 2013, Holmes et al., 2000). This behaviour has been reported in wild mice, particularly males, in the EPM (Shoji et al., 2016), and rather than indicating a high level of anxiety it may represent another approach of the mice, namely to identify and explore the most likely route of escape via the open arms. This hypothesis is partially supported by the previous finding that aged animals have higher plasma corticosterone levels than younger animals after exposure to a novel environment or a sudden noise (Takao et al., 2013, Herman et al., 2001, Kucuk et al., 2008). Taken together, the current findings on locomotion/activity, exploratory- and anxiety-related behaviours suggest that the two sexes adopt different behavioural strategies in response to novel, potentially stressful environments which depend on the specific nature of the challenge and the age of the mice.

Effect of repeated exposure to a behavioural task on performance

Many rodent behavioural tasks rely on the test subject responding to novelty in their environment. An often overlooked confounding factor in behavioural testing is repeat exposure/re-testing. In the current study, a 3-4 week period between behavioural testing influenced behavioural outcomes in a sex-dependant manner. In the OF test, reduced locomotor activity and time spent in the centre of the OF were observed in re-tested male and female mice, respectively. In the EPM, male mice with previous exposure to the maze showed a decrease in rearing upon re-exposure. Previous experience with the EPM is known to increase anxiety and diminish the effect of anxiolytic drugs, such as benzodiazepines, on second exposure to the task. This phenomenon termed “one trial tolerance” (File et al., 1990) is suggested to arise from the development of a phobic state after exposure to the EPM rather than an unconditioned anxiety response (File et al., 1993). Reduced motivation to explore may arise from the mice perceiving no benefit to exploring the open arms as they know there is no reward or escape option, so entering the anxiety-inducing environment becomes less desirable than in their first exposure. While the one trial tolerance phenomenon in the EPM has been well studied (File and Zangrossi, 1993, File et al., 1990, Schneider et al., 2011), less attention has been paid to the effect of previous exposure on behaviours within the OF task. In addition, the published work on prior exposure has primarily used males, with little information on female outcomes. Repeat OF exposure with a 24- hour interval, was shown to increase locomotion on the second day of testing in C57BL/6J mice (Fraser et al., 2010). Conversely, repeat testing of rats in the OF arena over a 10-day period led to an initial decrease in locomotion, followed by an increase back to initial levels (Williams and Russell, 1972). Although studies of

multiple exposures to the EPM in rodents have suggested that 3-4 weeks is a sufficient 'wash-out' period between tests, it appears that nuanced sex-specific effects in behaviour persist within this timeframe using C57BL/6J mice re-exposed to both the OF and EPM tasks.

Effect of a HFHS diet on food intake, growth rate, fat deposition and behaviour

There is a growing body of evidence from both human epidemiologic and animal experimental studies linking the consumption of diets high in fat or high in both fat and sugar, with abnormal brain morphology and adverse neurodevelopmental and psychiatric morbidity (Edlow, 2017, Contu and Hawkes, 2017, Godfrey et al., 2017). In human longitudinal studies, it is difficult to disentangle the influence of the maternal environment from the postnatal environment, as the diet in childhood and adolescence is likely to reflect the diet of the mother during pregnancy. The available literature indicates a commonality between the behaviour influenced by maternal obesity and postnatal obesity. Few studies, however, have investigated the effects of an obesogenic diet provided from post-weaning to adulthood. In the current study, post-weaning consumption of a HFHS diet led to the expected increases in body mass and adiposity in both male and female mice, with a 2-3 fold increment in total body fat mass and even greater percentage increases in the individual visceral fat deposits. These increases in body mass and fat accumulation occurred despite the mice consuming fewer kilocalories than their control-fed counterparts. Previous studies of rodents fed obesogenic diets in which food intake was measured have shown both increases and decreases in daily kilocalorie consumption (Wang et al., 2016, Haleem and Mahmood, 2021, Zanini et al., 2017). Increased adiposity is likely to increase the expression of leptin, which, in turn, would act to decrease appetite and reduce food intake (Fried et al., 2000), consistent with the current finding of reduced food intake by the mice accumulating more fat on the HFHS diet. In turn, the reduced food and protein intake may account for the reduced lean mass observed in both the male and female mice fed the HFHS diet. With less lean mass, the mice fed the HFHS diet are likely to have a reduced metabolic rate and energy expenditure, which would contribute to a lower kilocalorie requirement. Future studies are needed of the overall metabolic rate, and of the concentration of appetite and metabolism regulatory hormones, to understand how the HFHS diet leads to altered energy intake and body composition.

Consumption of a HFHS diet altered behaviour in both male and female mice. In the OF task, both male and female mice showed reduced locomotion, providing evidence of lower innate activity in the fatter mice, which would contribute to a lower overall energy expenditure. A variety of outcomes have been reported regarding locomotion/activity in diet-induced obesity models. Studies that fed mice a high fat diet or a high sucrose only diet, have reported either no change or increased locomotion (Eudave et al., 2018, Sharma and Fulton, 2013). Reduced locomotion within the OF task has been reported in obese mice (Takao et al., 2013, Buchenauer et al., 2009), consistent with the current results. This reduction in locomotion may be due to the increased body mass of the mice, less lean muscle mass or reduced motivation to explore the surrounding novel environment.

In people with a higher BMI, depression is more prevalent than in those in the healthy BMI range (Strine et al., 2008, Luppino et al., 2010). Moreover, depressive behaviours have been reported in mice fed a high fat diet (Yamada et al., 2011). In the current study, female mice fed the HFHS diet spent more time within the open arms, suggesting they were comfortable exploring the normally anxiogenic open arm environment. Furthermore, both males and females fed the HFHS diet showed increased visits to the distal end of the open arm, suggesting either reduced anxiety or increased motivation. Diet-induced postnatal obesity has been reported to induce anxiety in experimental animals in some, but not all studies using novelty suppressed feeding, OF tasks, and elevated zero maze (Zemdegs et al., 2016, Eudave et al., 2018, Akter et al., 2020, Xu et al., 2018, Almeida-Suhett et al., 2017, Bocarsly et al., 2015). Consistent with the current findings, rats and mice fed a high fat diet display reduced anxiety-related behaviour within the OF and EPM tasks (Haleem and Mahmood, 2021, Xu et al., 2018). These published studies only used male animals, and so differ from the current study where reduced anxiety-related behaviours were evident predominantly in female mice. Opposing anxiety behaviour outcomes within a published mouse obesity model have been reported (Cipriano et al., 2016) with increased anxiety-related behaviours evident in the OF task, but not in the elevated zero maze (Eudave et al., 2018).

Few experimental studies have examined the effects of a post-weaning diet high in fat and sugar, on social behaviour as the majority of the research has primarily focused on the effects of maternal obesity on their adult offspring. In the current study, mice fed the HFHS diet showed an increased preference for interacting with a novel mouse rather than an object. This effect was evident in both sexes. A previous study has reported that a short, 10-day exposure to a high fat or high sucrose diet did not alter social behaviour (Eudave et al., 2018). However, a longer period of 8 weeks on a high fat diet led to increased social interaction in male rats (Buchenauer et al., 2009), which supports our finding of increased social interaction after 9 weeks on the HFHS diet. In the current study, the increase in sociability observed in the SP task combined with the increased number of visits to the distal end of the open arm of the EPM, may be indicative of increased risk-taking or novelty-seeking behaviour (Shoji and Miyakawa, 2021, Ludwig et al., 2021). Further studies are therefore needed to establish the true cause of this apparent increased sociability in the mice fed the HFHS diet. In summary, in the current study, post-weaning consumption of an obesogenic diet high in both fat and sugar, led to a significant reduction in locomotion/activity in both sexes, differential effects on anxiety-related behaviours to height and open spaces in females only, while social preference was significantly increased in both male and female mice. This data shows that diet-induced obesity exclusively in postnatal life leads to altered behaviours in both males and females.

Conclusions

The current results show that many aspects of mouse behaviour are sex-specific. The effects of age, prior exposure to a behaviour test and feeding an obesogenic diet, on locomotion and/or anxiety behaviours all depended on the sex of the mouse. Males and females appeared to adopt different behavioural strategies in coping with novel, stressful conditions. These responses also differed depending on their diet and on whether the anxiety was induced by the height of the EPM or the open space within the OF arena. Overall, the findings highlight the role of diet, food intake, body composition and age of the mice on mouse behaviour. The results also emphasise the importance of including both sexes in behavioural studies.

References

AKTER, S., UDDIN, K. R., SASAKI, H. & SHIBATA, S. 2020. Gamma Oryzanol Alleviates High-Fat Diet-Induced Anxiety-Like Behaviors Through Downregulation of Dopamine and Inflammation in the Amygdala of Mice. *Front Pharmacol,* 11**,** 330.

ALMEIDA-SUHETT, C. P., GRAHAM, A., CHEN, Y. & DEUSTER, P. 2017. Behavioral changes in male mice fed a high-fat diet are associated with IL-1beta expression in specific brain regions. *Physiol Behav,* 169**,** 130-140.

BERTOGLIO, L. J. & CAROBREZ, A. P. 2000. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behav Brain Res,* 108**,** 197-203.

BOCARSLY, M. E., FASOLINO, M., KANE, G. A., LAMARCA, E. A., KIRSCHEN, G. W., KARATSOREOS, I. N., MCEWEN, B. S. & GOULD, E. 2015. Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. *Proc Natl Acad Sci U S A,* 112**,** 15731-6.

BUCHENAUER, T., BEHRENDT, P., BODE, F. J., HORN, R., BRABANT, G., STEPHAN, M. & NAVE, H. 2009. Diet-induced obesity alters behavior as well as serum levels of corticosterone in F344 rats. *Physiol Behav,* 98**,** 563-9.

CIPRIANO, A. C., GOMES, K. S. & NUNES-DE-SOUZA, R. L. 2016. CRF receptor type 1 (but not type 2) located within the amygdala plays a role in the modulation of anxiety in mice exposed to the elevated plus maze. *Horm Behav,* 81**,** 59-67.

CLARK, T. D., CREAN, A. J. & SENIOR, A. M. 2022a. Obesogenic diets induce anxiety in rodents: A systematic review and meta-analysis. *Obes Rev,* 23**,** e13399.

CLARK, T. D., REICHELT, A. C., GHOSH-SWABY, O., SIMPSON, S. J. & CREAN, A. J. 2022b. Nutrition, anxiety and hormones. Why sex differences matter in the link between obesity and behavior. *Physiol Behav,* 247**,** 113713.

CONTU, L. & HAWKES, C. A. 2017. A Review of the Impact of Maternal Obesity on the Cognitive Function and Mental Health of the Offspring. *Int J Mol Sci,* 18.

DAVIS, M. J., HALEY, T., DUVOISIN, R. M. & RABER, J. 2012. Measures of anxiety, sensorimotor function, and memory in male and female mGluR4(-)/(-) mice. *Behav Brain Res,* 229**,** 21-8.

NHS DIGITAL. 2020. *Statistics on Obesity, Physical Activity and Diet, England*. https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-obesity-physical-activity-and-diet. [Accessed 15 February 2023].

EDLOW, A. G. 2017. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. *Prenat Diagn,* 37**,** 95-110.

EUDAVE, D. M., BELOW, M. N. & FLANDREAU, E. I. 2018. Effects of high fat or high sucrose diet on behavioral-response to social defeat stress in mice. *Neurobiol Stress,* 9**,** 1-8.

FILE, S. E., MABBUTT, P. S. & HITCHCOTT, P. K. 1990. Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. *Psychopharmacology (Berl),* 102**,** 98-101.

FILE, S. E. & ZANGROSSI, H., JR. 1993. "One-trial tolerance" to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? *Psychopharmacology (Berl),* 110**,** 240-4.

FILE, S. E., ZANGROSSI, H., JR., VIANA, M. & GRAEFF, F. G. 1993. Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology (Berl),* 111**,** 491-4.

FRASER, L. M., BROWN, R. E., HUSSIN, A., FONTANA, M., WHITTAKER, A., O'LEARY, T. P., LEDERLE, L., HOLMES, A. & RAMOS, A. 2010. Measuring anxiety- and locomotion-related behaviours in mice: a new way of using old tests. *Psychopharmacology (Berl),* 211**,** 99-112.

FRIED, S. K., RICCI, M. R., RUSSELL, C. D. & LAFERRERE, B. 2000. Regulation of leptin production in humans. *J Nutr,* 130**,** 3127S-3131S.

FULTON, S., DECARIE-SPAIN, L., FIORAMONTI, X., GUIARD, B. & NAKAJIMA, S. 2022. The menace of obesity to depression and anxiety prevalence. *Trends Endocrinol Metab,* 33**,** 18-35.

GANGITANO, D., SALAS, R., TENG, Y., PEREZ, E. & DE BIASI, M. 2009. Progesterone modulation of alpha5 nAChR subunits influences anxiety-related behavior during estrus cycle. *Genes Brain Behav,* 8**,** 398-406.

GODFREY, K. M., REYNOLDS, R. M., PRESCOTT, S. L., NYIRENDA, M., JADDOE, V. W., ERIKSSON, J. G. & BROEKMAN, B. F. 2017. Influence of maternal obesity on the long-term health of offspring. *Lancet Diabetes Endocrinol,* 5**,** 53-64.

GRIEBEL, G., BELZUNG, C., PERRAULT, G. & SANGER, D. J. 2000. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology (Berl),* 148**,** 164-70.

HAGAN, C. 2017. *When are mice considered old?* https://www.jax.org/news-and-insights/jax-blog/2017 https://www.jax.org/news-and-insights/jax-blog/2017/November/when-are-mice-considered-old. [Accessed 15 February 2023].

HALEEM, D. J. & MAHMOOD, K. 2021. Brain serotonin in high-fat diet-induced weight gain, anxiety and spatial memory in rats. *Nutr Neurosci,* 24**,** 226-235.

HATTORI, S., TAKAO, K., TANDA, K., TOYAMA, K., SHINTANI, N., BABA, A., HASHIMOTO, H. & MIYAKAWA, T. 2012. Comprehensive behavioral analysis of pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice. *Front Behav Neurosci,* 6**,** 58.

HERMAN, J. P., LARSON, B. R., SPEERT, D. B. & SEASHOLTZ, A. F. 2001. Hypothalamo-pituitary-adrenocortical dysregulation in aging F344/Brown-Norway F1 hybrid rats. *Neurobiol Aging,* 22**,** 323-32.

HOLMES, A., PARMIGIANI, S., FERRARI, P. F., PALANZA, P. & RODGERS, R. J. 2000. Behavioral profile of wild mice in the elevated plus-maze test for anxiety. *Physiol Behav,* 71**,** 509-16.

KUCUK, A., GOLGELI, A., SARAYMEN, R. & KOC, N. 2008. Effects of age and anxiety on learning and memory. *Behav Brain Res,* 195**,** 147-52.

LALONDE, R. & STRAZIELLE, C. 2009. Exploratory activity and motor coordination in old versus middle-aged C57BL/6J mice. *Arch Gerontol Geriatr,* 49**,** 39-42.

LOPEZ-TABOADA, I., GONZALEZ-PARDO, H. & CONEJO, N. M. 2020. Western Diet: Implications for Brain Function and Behavior. *Front Psychol,* 11**,** 564413.

LUDWIG, M., RICHTER, M., GOLTERMANN, J., REDLICH, R., REPPLE, J., FLINT, C., GROTEGERD, D., KOCH, K., LEEHR, E. J., MEINERT, S., HULSMANN, C., ENNEKING, V., KUGEL, H., HAHN, T., BAUNE, B. T., DANNLOWSKI, U. & OPEL, N. 2021. Novelty seeking is associated with increased body weight and orbitofrontal grey matter volume reduction. *Psychoneuroendocrinology,* 126**,** 105148.

LUPPINO, F. S., DE WIT, L. M., BOUVY, P. F., STIJNEN, T., CUIJPERS, P., PENNINX, B. W. & ZITMAN, F. G. 2010. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry,* 67**,** 220-9.

MACRI, S., ADRIANI, W., CHIAROTTI, F. & LAVIOLAF, G. 2003. Risk taking during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. *Anim Behav* 64**,** 541-546.

MARCONDES, F. K., MIGUEL, K. J., MELO, L. L. & SPADARI-BRATFISCH, R. C. 2001. Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiol Behav,* 74**,** 435-40.

MIYAKAWA, T., LEITER, L. M., GERBER, D. J., GAINETDINOV, R. R., SOTNIKOVA, T. D., ZENG, H., CARON, M. G. & TONEGAWA, S. 2003. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc Natl Acad Sci U S A,* 100**,** 8987-92.

MOY, S. S., NADLER, J. J., PEREZ, A., BARBARO, R. P., JOHNS, J. M., MAGNUSON, T. R., PIVEN, J. & CRAWLEY, J. N. 2004. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav,* 3**,** 287-302.

NAPSO, T., LEAN, S. C., LU, M., MORT, E. J., DESFORGES, M., MOGHIMI, A., BARTELS, B., EL-BACHA, T., FOWDEN, A. L., CAMM, E. J. & SFERRUZZI-PERRI, A. N. 2022. Diet-induced maternal obesity impacts feto-placental growth and induces sex-specific alterations in placental morphology, mitochondrial bioenergetics, dynamics, lipid metabolism and oxidative stress in mice. *Acta Physiol (Oxf),* 234**,** e13795.

NOLTE, E. D., NOLTE, K. A. & YAN, S. S. 2019. Anxiety and task performance changes in an aging mouse model. *Biochem Biophys Res Commun,* 514**,** 246-251.

WORLD HEALTH ORGANISATION. 2021. *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. [Accessed 15 February 2023].

POWELL-WILEY, T. M., POIRIER, P., BURKE, L. E., DESPRES, J. P., GORDON-LARSEN, P., LAVIE, C. J., LEAR, S. A., NDUMELE, C. E., NEELAND, I. J., SANDERS, P., ST-ONGE, M. P., AMERICAN HEART ASSOCIATION

COUNCIL ON, L., CARDIOMETABOLIC, H., COUNCIL ON, C., STROKE, N., COUNCIL ON CLINICAL, C., COUNCIL ON, E., PREVENTION & STROKE, C. 2021. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation,* 143**,** e984-e1010.

PRUT, L. & BELZUNG, C. 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol,* 463**,** 3-33.

RATCLIFF, M. B., JENKINS, T. M., REITER-PURTILL, J., NOLL, J. G. & ZELLER, M. H. 2011. Risk-taking behaviors of adolescents with extreme obesity: normative or not? *Pediatrics,* 127**,** 827-34.

RODGERS, R. J. & SHEPHERD, J. K. 1993. Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. *Psychopharmacology (Berl),* 113**,** 237-42.

SCHNEIDER, P., HO, Y. J., SPANAGEL, R. & PAWLAK, C. R. 2011. A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. *Front Behav Neurosci,* 5**,** 43.

SHARMA, S. & FULTON, S. 2013. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int J Obes (Lond),* 37**,** 382-9.

SHOJI, H. & MIYAKAWA, T. 2021. Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: a challenge against conventional interpretation of the test. *Mol Brain,* 14**,** 34.

SHOJI, H., TAKAO, K., HATTORI, S. & MIYAKAWA, T. 2016. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain,* 9**,** 11.

STRINE, T. W., MOKDAD, A. H., DUBE, S. R., BALLUZ, L. S., GONZALEZ, O., BERRY, J. T., MANDERSCHEID, R. & KROENKE, K. 2008. The association of depression and anxiety with obesity and unhealthy behaviors among community-dwelling US adults. *Gen Hosp Psychiatry,* 30**,** 127-37.

STURMAN, O., GERMAIN, P. L. & BOHACEK, J. 2018. Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. *Stress,* 21**,** 443-452.

TAKAO, K., KOBAYASHI, K., HAGIHARA, H., OHIRA, K., SHOJI, H., HATTORI, S., KOSHIMIZU, H., UMEMORI, J., TOYAMA, K., NAKAMURA, H. K., KUROIWA, M., MAEDA, J., ATSUZAWA, K., ESAKI, K., YAMAGUCHI, S., FURUYA, S., TAKAGI, T., WALTON, N. M., HAYASHI, N., SUZUKI, H., HIGUCHI, M., USUDA, N., SUHARA, T., NISHI, A., MATSUMOTO, M., ISHII, S. & MIYAKAWA, T. 2013. Deficiency of schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and confers molecular, neuronal, and behavioral phenotypes related to schizophrenia. *Neuropsychopharmacology,* 38**,** 1409-25.

WALF, A. A. & FRYE, C. A. 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols,* 2**,** 322-328.

WANG, S., HUANG, X. F., ZHANG, P., WANG, H., ZHANG, Q., YU, S. & YU, Y. 2016. Chronic rhein treatment improves recognition memory in high-fat diet-induced obese male mice. *J Nutr Biochem,* 36**,** 42-50.

WILLIAMS, D. I. & RUSSELL, P. A. 1972. Open-field behaviour in rats: effects of handling sex and repeated testing. *Br J Psychol,* 63**,** 593-6.

XU, L., XU, S., LIN, L., GU, X., FU, C., FANG, Y., LI, X. & WANG, X. 2018. High-fat Diet Mediates Anxiolytic-like Behaviors in a Time-dependent Manner Through the Regulation of SIRT1 in the Brain. *Neuroscience,* 372**,** 237-245.

YAMADA, N., KATSUURA, G., OCHI, Y., EBIHARA, K., KUSAKABE, T., HOSODA, K. & NAKAO, K. 2011. Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology,* 152**,** 2634-43.

YOSHIZAKI, K., ASAI, M. & HARA, T. 2020. High-Fat Diet Enhances Working Memory in the Y-Maze Test in Male C57BL/6J Mice with Less Anxiety in the Elevated Plus Maze Test. *Nutrients,* 12.

ZANINI, P., ARBO, B. D., NICHES, G., CZARNABAY, D., BENETTI, F., RIBEIRO, M. F. & CECCONELLO, A. L. 2017. Diet-induced obesity alters memory consolidation in female rats. *Physiol Behav,* 180**,** 91-97.

ZEMDEGS, J., QUESSEVEUR, G., JARRIAULT, D., PENICAUD, L., FIORAMONTI, X. & GUIARD, B. P. 2016. High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. *Br J Pharmacol,* 173**,** 2095-110.

Figure Legends

Figure 1: Age and sex differences in mouse behaviour in the OF.

A. Locomotor activity in the OF measured as the number of lines crossed for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p<0.0001$, ANOVA). **B**. Effect of age on the number of lines crossed in male mice ($p<0.0001$, ANOVA). **C**. Number of lines crossed in female mice ($p=0.02$, ANOVA). **D**. Open space anxiety-related behaviour in the OF measured as the number of entries into the centre for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.0001$, Kruskal Wallis). **E**. Number of entries into the centre in male mice ($p=0.002$, ANOVA). **F**. Number of entries into the centre in female mice ($p=0.08$, Kruskal Wallis). **G**. Time (seconds) spent in the centre of the OF for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.46$, Kruskal Wallis). **H, I**. There were no significant age effects in male ($p=0.49$, Kruskal Wallis) or female ($p=0.72$, Kruskal Wallis) mice. **J**. Exploratory rearing in the OF in all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.017$, Kruskal Wallis). **K**. There was a significant effect of age on the number of rears in male mice ($p=0.012$, Kruskal Wallis). **L**. There was no effect of age in female mice ($p=0.17$, ANOVA). Numbers of mice (see Methods): 8-9 weeks, 13 males and 13 females; 11-13 weeks, 14 males and 11 females; 20-22 weeks, 18 males and 5 females. Post-hoc tests (see Methods): *$p<0.05$; **$p<0.001$; ***$p<0.0001$.

Figure 2: Age and sex differences in mouse behaviour in the EPM.

A. Height anxiety-related behaviour in the EPM measured as percentage time spent in the open arm for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.0002$, ANOVA). **B**. Percentage time in the open arm in male mice ($p=0.0006$, ANOVA). **C**. Percentage time in the open arm in female mice ($p=0.042$, ANOVA). **D**. Full entries into the open arm of the EPM, a measure of both anxiety and locomotion, was tested in all mice at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.0001$, Kruskal Wallis). **E**. Full entries to the open arm in male mice ($p=0.008$, ANOVA). **F**. Full entries to the open arm in female mice ($p=0.01$, ANOVA). **G**. Full entries into the closed arm, a measure of locomotion in the EPM, for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.0003$, ANOVA). **H**. Full entries into the closed arm in male mice ($p=0.013$, ANOVA). **I**. Full entries into the closed arm in female mice ($p=0.017$, Kruskal Wallis). **J**. Explorations to the end of the open arm for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.65$, Kruskal Wallis). **K**. End of open arm explorations in male mice ($p=0.26$, ANOVA). **L**. End of open arm explorations in female mice ($p=0.025$, Kruskal Wallis). **M**. Total rearing for all mice tested at either 8-9 weeks, 11-13 weeks or 20-22 weeks ($p=0.0002$, Kruskal Wallis). **N**. Rearing in male mice ($p<0.0001$, Kruskal Wallis). **P**. Rearing in female mice ($p=0.21$, Kruskal Wallis). Numbers of mice (see Methods): 8-9 weeks, 13 males and 13 females; 11-13 weeks, 15 males and 12 females; 20-22 weeks, 18 males and 5 females. Post-hoc tests (see Methods): *$p<0.05$; **$p<0.001$; ***$p<0.0001$.

Figure 3: Repeated experience of the OF has limited effects on behaviour

A. The number of lines crossed by male mice (11-13 weeks) in the OF for the first time(naïve) versus male mice (11-13 weeks) that were previously tested in the OF at 8-9 weeks (exposed; **p<0.001, t-test). **B**. Lines crossed by naïve versus exposed female mice (11-13 weeks) in the OF (p=0.15, t-test). **C,D**. Entries into the centre of the OF by naïve and exposed 11-13 week old male (p=0.24, t-test) and female (p=0.42, Mann Whitney) mice. **E,F**. Time (seconds) spent in the centre of the OF by naïve and exposed 11-13 week old male (p=0.9, Mann Whitney) and female (*p<0.05, Mann Whitney) mice. **G,H**. Number of rears made in the OF by naïve and exposed 11-13 week old male (p=0.15, t-test) and female (p=0.122, Mann Whitney) mice. Numbers of mice: naïve, 14 males and 11 females; exposed, 13 males and 13 females.

Figure 4: Repeated experience of the EPM has limited effects on behaviour

A. The percentage time spent in the open arm of the EPM by male mice (11-13 weeks) for the first time (naïve) versus male mice (11-13 weeks) that were previously tested in the EPM at 8-9 weeks (exposed; p=0.09, t-test). **B**. Percentage time in the open arm for naïve versus exposed female mice (11-13 weeks; p=0.06, t-test). **C,D**. Number of full entries into the open arm by naïve and exposed 11-13 week old male (p=0.28, t-test) and female (p=0.78, t-test) mice. **E,F**. Number of full entries into the closed arm by naïve and exposed 11-13 week old male (p=0.45, t-test) and female (p=0.14, t-test) mice. **G,H**. Number of explorations to the end of the open arm by naïve and exposed 11-13 week old male (p=0.11, t-test) and female (p=0.13, Mann Whitney) mice. **I,J**. Number of rears by naïve and exposed 11-13 week old male (p=0.007, Mann Whitney) and female (p=0.077, t-test) mice. Numbers of mice: naïve, 15 males and 12 females; exposed, 13 males and 13 females.

Figure 5 An obesogenic diet alters nutrient intake in male and female mice

A. Food intake (gram of food per day per gram of mouse) in male mice fed either a control diet (filled bars) or a high fat/ high sugar (HFHS) diet (open bars) from weaning, measured between 10-13 weeks. **B**. Food intake (gram of food per day per gram of mouse) in female mice fed either a control diet or a HFHS diet from weaning, measured between 10-13 weeks. 2-way ANOVA; posthoc test of diet effect, *p<0.05, **p<0.01, ***p<0.001. **C**. Kilocalorie intake (kcal per day per gram of mouse) in male mice fed either a control diet or a HFHS diet from weaning, measured between 10-13 weeks. Mixed effects analysis; posthoc test of diet effect, *p<0.05, **p<0.01. **D**. Kilocalorie intake (kcal per day per gram of mouse) in female mice fed either a control diet or a HFHS diet from weaning, measured between 10-13 weeks. 2-way ANOVA; posthoc test of diet effect, *p<0.05. **E**. Protein intake (gram of protein per day per gram of mouse) in male mice fed either a control diet or a HFHS diet from weaning, measured between 10-13 weeks. Mixed effects analysis; posthoc test of diet effect, ***p<0.001. **F**. Protein intake (gram of protein per day per gram of mouse) in female mice fed either a control diet or a HFHS diet from weaning, measured between 10-13 weeks. 2-way ANOVA; posthoc test of diet effect,

***p<0.001. Numbers of cages of mice: control diet, 5 cages of males, 5 cages of females; HFHS diet, 7-8 cages of males, 10 cages of females.

Figure 6: An obesogenic diet affects growth and adiposity in male and female mice

A. Body mass (g) of male mice on either a control (n=14-19) or HFHS (n=13-14) diet from weaning until postnatal day 98. Mixed effects analysis; *post hoc* test of diet effect, *p<0.05, **p<0.01. **B**. Body mass (g) of female mice on either a control (n=16-18) or HFHS (n=14) diet from weaning until postnatal day 98. *Post hoc* test of diet effect, **p<0.01, ***p<0.001. **C**. Example DEXA scans of male mice aged 98 days on the control diet (left) or HFHS diet (right). **D**. Example DEXA scans of female mice aged 98 days on the control diet (left) or HFHS diet (right). **E**. Fat mass (g) and **F**. lean mass (g) of male mice on the control (n=8) or HFHS (n=6) diet, determined by DEXA (**p=0.005, t-test; p=0.002, t-test). **G**. Fat mass (g) and **H**. lean mass (g) of female mice on the control (n=13) or HFHS (n=8) diet, determined by DEXA (***p<0.001, t-test). Post-mortem measurements of organ fat deposits following a control or HFHS diet: **I**. Perirenal fat in male mice (***p=0.0002, t-test; n=13-15). **J**. Retroperitoneal fat in male mice (***p<0.0001, t-test; n=13-15). **K**. Perirenal fat in female mice (***p<0.0001, t-test; n=13-20). **L**. Retroperitoneal fat in female mice (***p<0.0001, t-test; n=13-20). **M, O**. Gonadal fat in male (***p<0.0001, t-test; n=11-13) and female mice (***p<0.0001, t-test; n=9-13). **N**. Organs fat expressed as a percentage of total fat measured post-mortem in male mice (perirenal, mid-grey; retroperitoneal, dark grey; gonadal, white; other, pale grey). **P**. Organs fat expressed as a percentage of total fat measured post-mortem in female mice (colour code as for male mice in N). Data in N and P were arcsin transformed for statistical analysis of fat deposits in HFHS versus control diet (perirenal fat males, p=0.015, females, p=0.08; gonadal fat males, p=0.002, females, p=0.14; retroperitoneal fat males, p=0.0004, females, p=0.002; t-test.

Figure 7: An obesogenic diet affects locomotor but not anxiety behaviour in the OF

A. The number of lines crossed in the OF by male mice (11-13 weeks) on a control or HFHS diet (**p=0.001, t-test). **B**. Lines crossed by female mice (11-13 weeks) on a control or HFHS diet (*p=0.011, Mann Whitney). **C,D**. Entries into the centre of the OF by 11-13 week old male (p=0.06, t-test) and female (p=0.09, Mann Whitney) mice on a control versus a HFHS diet. **E,F**. Time (seconds) spent in the centre of the OF by 11-13 week old male (p=0.17, Mann Whitney) and female (p=0.54, Mann Whitney) mice on a control or HFHS diet. **G,H**. Number of rears made in the OF by 11-13 week old male (p=0.83, t-test) and female (p=0.85, t-test) mice on a control or HFHS diet. Numbers of mice: control diet, 30 males and 26 females; HFHS diet, 13 males and 11 females.

Figure 8: Sex differences in the effect of an obesogenic diet in the EPM

A. The percentage time spent in the open arm of the EPM by male mice (11-13 weeks) on a control or HFHS diet (p=0.57, t-test). **B**. Percentage time in the open arm for female mice (11-13 weeks) on a control or HFHS diet (***p=0.0003, t-test). **C,D**. Number of full entries into the open arm by 11-13 week old male (p=0.81, t-test) and female (*p=0.04, Mann Whitney) mice on a control or HFHS diet. **E,F**. Number of full entries into the closed arm by 11-13 week old male (p=0.96, t-test) and female (**p=0.009, t-test) mice on a control or HFHS diet. **G,H**. Number of explorations to the end of the open arm by 11-13 week old male (*p=0.048, t-test) and female (***p<0.0001, Mann Whitney) mice on a control or HFHS diet. Numbers of mice: control diet, 30 males and 26 females; HFHS diet, 12 males and 14 females.

Figure 9: An obesogenic diet affects social interaction behaviour in male and female mice

A. Male mice on the HFHS diet (n=13) spent a significantly greater percentage time in the intruder compartment versus the object compartment than male mice on the control diet (n=15; **p=0.004, t-test). **B**. Female mice on the HFHS diet (n=12) spent a significantly greater percentage time in the intruder compartment versus the object compartment than male mice on the control diet (n=15; **p=0.03, t-test). **C**. The social preference index, a measure of the time spent interacting with the intruder mouse versus the object, was significantly increased in male mice (p=0.007, t-test). **D**. The social preference index was significantly increased in female mice (p=0.0002, t-test).